HORTON, ROBERT J. M., M.D.—Chief, Field Studies Branch, Division of Air Pollution, U.S. Public Health Service, Cincinnati, Ohio.

HUEPER, WILHELM C., M.D.—Chief, Environmental Cancer Section, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.

IPSEN, JOHANNES, Ph. D.—Professor of Medical Statistics, Henry Phipps Institute, University of Pennsylvania, Philadelphia, Pa.

ISBELL, HARRIS, M.D.—Professor of Clinical Pharmacology, University of Kentucky Medical School, Lexington, Ky.

ISKRANT, ALBERT P.—Chief, Developmental Research Section, Division of Accident Prevention, U.S. Public Health Service, Washington, D.C.

JANUS, ZELDA—Statistician, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.

JOSIE, G. H., Sc. D., M.P.H.—Chief, Epidemiology Division, Department of National Health and Welfare, Ottawa, Canada.

KAHN, HAROLD A.—Statistician, Biometrics Research Branch, National Heart Institute, U.S. Public Health Service, Bethesda, Md.

KANNEL, W. B., M.D.—Associate Director, Heart Disease Epidemiology Study, National Heart Institute, U.S. Public Health Service, Framingham, Mass.

KELEMEN, GEORGE, M.D.—Research Associate, Massachusetts Eye and Ear Infirmary, Harvard University Medical School, Boston, Mass.

KELLEY, HAROLD H., Ph. D.—Professor, Department of Psychology, University of California, Los Angeles, Calif.

KENSLER, CHARLES J., Ph. D.—Senior Vice President, Life Sciences Division, Arthur D. Little, Inc., Cambridge, Mass.

KESSELMAN, AVIVA—Statistician, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.

KLEINERMAN, JEROME, M.D.—Associate Director, Medical Research Department, St. Luke's Hospital, Cleveland, Ohio

KNIGHT, VERNON, M.D.—Clinical Director, National Institute of Allergy and Infectious Diseases, U.S. Public Health Service, Bethesda, Md.

KNUTTI, RALPH E., M.D.—Director, National Heart Institute, U.S. Public Health Service, Bethesda, Md.

KOTIN, PAUL, M.D.—Associate Director of Field Studies, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.

KREYBERG, LEIV, M.D.—Director of Institute for General and Experimental Pathology, University of Oslo, Oslo, Norway

KRUEGER, DEAN E.—Statistician, Biometrics Research Branch, National Heart Institute, U.S. Public Health Service, Bethesda, Md.

KUSCHNER, MARVIN, M.D.—Professor of Pathology and Director of Laboratories, Bellevue Hospital Center, New York University Medical Center, New York, N.Y.

LARSON, PAUL S., Ph. D.—Professor and Chairman of Department of Pharmacology, Medical College of Virginia, Richmond, Va.

LEITER, JOSEPH, Ph. D.—Chief, Cancer Chemotherapy National Service Center, U.S. Public Health Service, Silver Spring, Md.

LEUCHTENBERGER, CECILIE, M.D., Ph. D.—Professor, Eidgenössische Technische Hochschule, Institut für Allgemeine Botanik, Zurich, Switzerland

XIII

GOLDSTEIN, HYMAN, Ph. D.—Chief, Biometrics Branch, National Institute of Neurological Diseases and Blindness, U.S. Public Health Service, Bethesda, Md.

GRAHAM, SAXON, M.D.—Associate Cancer Research Scientist, Roswell Park Memorial Institute, Buffalo, N.Y.

GREENBERG, BERNARD G., Ph. D.—Professor of Biostatistics, School of Public Health, University of North Carolina, Chapel Hill, N.C.

GROSS, PAUL, M.D.—Research Pathologist, Industrial Hygiene Foundation, Mellon Institute, Pittsburgh, Pa.

HAENSZEL, WILLIAM—Chief, Biometry Branch, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.

HAINER, RAYMOND M., Ph. D.—Research Physical Chemist, A. D. Little Inc., Cambridge, Mass.

HALL, ROBERT L., Ph. D.—Program Director, Sociology and Social Psychology, National Science Foundation, Washington, D.C.

HALMSTAD, DAVID—Actuary, The National Center for Health Statistics, U.S. Public Health Service, Washington, D.C.

HAMMOND, E. CUYLER, Sc. D.—Director, Statistical Research Section, Medical Affairs Department, American Cancer Society, Inc., New York, N.Y.

HAMPERL, H., M.D.—Director of the Pathology Institute, University of Bonn, Bonn, Germany.

HARTWELL, JONATHAN L., Ph. D.—Chief, Research Communications Branch, National Cancer Institute, U.S. Public Health Service, Silver Spring, Md.

HAYDEN, ROBERT G., Ph. D.—Research Psychologist, Behavioral Sciences Section, Division of Community Health Services, U.S. Public Health Service, Washington, D.C.

HEIMANN, HARRY, M.D.—Chief, Division of Occupational Health, U.S. Public Health Service, Washington, D.C.

HEINZELMANN, FRED, Ph. D.—Assistant Chief, Behavioral Sciences Section, Division of Community Health Services, U.S. Public Health Service, Washington, D.C.

HELLER, JOHN R., Jr., M.D.—President and Chief Executive Officer, Sloan-Kettering Institute for Cancer Research, New York, N.Y.

HERMAN, DORIS L., M.D.—Pathologist, Tumor Tissue Registry, Cancer Commission, California Medical Association, Los Angeles, Calif.

HERROLD, KATHERINE, M.D.—Medical Director, Laboratory of Pathology, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.

HESTON, WALTER E., M.D., Ph. D.—Chief, Laboratory of Biology, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.

HIGGINS, IAN T. T., M.D.—Professor of Epidemiology and Microbiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, Pa.

HOCHBAUM, GODFREY, Ph. D.—Chief, Behavioral Sciences Section, Division of Community Health Services, U.S. Public Health Service, Washington, D.C.

HOCKETT, ROBERT C., Ph. D.—Associate Scientific Director, Tobacco Industry Research Committee, New York, N.Y.

HORN, DANIEL, Ph. D.—Assistant Chief for Research, Cancer Control Program, Division of Chronic Diseases, U.S. Public Health Service, Washington, D.C.

LEUCHTENBERGER, RUDOLF, M.D.—Professor Eidgenössische Technische Hochschule, Institut für Allgemeine Botanik, Zurich, Switzerland
LEVIN, MORTON L., M.D.—Professor of Epidemiology, Roswell Park Memorial Institute, Buffalo, N.Y.
LIEBOW, AVERILL A., M.D.—Professor of Pathology, Yale University School of Medicine, New Haven, Conn.
LIGGETT & MYERS, INC., New York, N.Y.
LILIENFELD, ABRAHAM, M.D.—Professor of Chronic Diseases, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Md.
LISCO, HERMAN, M.D.—Cancer Research Institute, New England Deaconess Hospital, Boston, Mass.
LITTLE, CLARENCE COOK, M.D.—Scientific Director, Tobacco Institute Research Committee, New York, N.Y.
LOUDON, R. G., M.B.—Assistant Professor of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Tex.
MANOS, NICHOLAS E.—Statistician, Division of Occupational Health, U.S. Public Health Service, Washington, D.C.
MARDER, MARTIN, Ph. D.—Research Psychologist, Behavioral Sciences Section, Division of Community Health Services, U.S. Public Health Service, Washington, D.C.
MATARAZZO, J. D., Ph. D.—Professor of Medical Psychology, Department of Medical Psychology, University of Oregon Medical School, Portland, Oreg.
McFARLAND, JAMES J., M.D.—Professor of Otolaryngology, School of Medicine, George Washington University Hospital, Washington, D.C.
McGILL, HENRY C., M.D.—Professor of Pathology, Louisiana State University School of Medicine, New Orleans, La.
McHUGH, RICHARD B., Ph. D.—Associate Professor of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, Minn.
McKENNIS, HERBERT, Jr.—Professor of Pharmacology, Medical College of Virginia, Richmond, Va.
MEDALIA, NAHUM Z., Ph. D.—Executive Secretary, Mental Health Small Grants Committee, National Institute of Mental Health, U.S. Public Health Service, Bethesda, Md.
MEHLER, MRS. ANN—Research Assistant, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.
MILLER, JACK, M.D.—Research Fellow in Medicine, The University of Texas Southwestern Medical School, Dallas, Tex.
MILLER, ROBERT W., M.D.—Chief, Epidemiology Section, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.
MILLER, WILLIAM F., M.D.—Associate Professor of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Tex.
MITCHELL, ROGER S., M.D.—Associate Professor, University of Colorado School of Medicine, Denver, Colo.
MURPHY, EDMOND A., M.D.—Attending Physician, The Moore Clinic, The Johns Hopkins University Hospital, Baltimore, Md.
NASH, HARVEY, Ph. D.—Illinois State Psychiatric Institute, Northwestern University Medical School, Chicago, Ill.

NELSON, NORTON, Ph. D.—Professor and Chairman, Department of Industrial Medicine, New York University Medical Center, New York, N.Y.
ORCHIN, MILTON, Ph. D.—Professor of Chemistry, University of Cincinnati, Cincinnati, Ohio.
P. LORILLARD CO., New York, N.Y.
PAFFENBARGER, RALPH S., Jr., M.D.—Medical Director, Field Epidemiology Research Section, National Heart Institute, U.S. Public Health Service, Framingham, Mass.
PAUL, OGLESBY, M.D.—Chairman, Committee on Epidemiological Studies, Passavant Memorial Hospital, Chicago, Ill.
PFAELZER, ANNE I.—Concord, Mass.
PHILLIP MORRIS, INC., New York, N.Y.
PICKREN, JOHN W., M.D.—Chief, Department of Pathology, Roswell Park Memorial Institute, Buffalo, N.Y.
PIERCE, JOHN A., M.D.—Associate Professor, Department of Medicine, University of Arkansas Medical Center, Little Rock, Ark.
POTTS, ALBERT M., M.D.—Professor of Ophthalmology, University of Chicago School of Medicine, Chicago, Ill.
PRINDLE, RICHARD A., M.D.—Chief, Division of Public Health Methods, U.S. Public Health Service, Washington, D.C.
R. J. REYNOLDS TOBACCO CO., Winston-Salem, N.C.
REED, SHELDON C., Ph. D.—Professor of Zoology, Department of Zoology, University of Minnesota, Minneapolis, Minn.
REMINGTON RAND, LTD. (Ottawa)
ROOS, CHARLES A.—Head, Reference Services Section, National Library of Medicine, U.S. Public Health Service, Bethesda, Md.
ROSEN, SAMUEL, M.D.—Chief, Pulmonary Mediastinal and ENT Pathology Branch, Armed Forces Institute of Pathology, Washington, D.C.
ROSENBLATT, MILTON B., M.D.—Associate Clinical Professor of Medicine, New York Medical College, and Visiting Physician, Metropolitan Hospital, New York, N.Y.
ROSS, JOSEPH, M.D.—Associate Professor of Medicine, University of Indiana School of Medicine and Head of Chest Division, Robert Long Hospital, Indianapolis, Ind.
SANFORD, J. P., M.D.—Associate Professor of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Tex.
SAVAGE, I. RICHARD, Ph. D.—Professor of Statistics, Florida State University, Tallahassee, Fla.
SCHIFFMAN, ZELDA—Special Assistant to Executive Officer, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.
SCHNEIDERMAN, MARVIN. A—Associate Chief, Biometry Branch, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.
SCHWARTZ, JOHN THEODORE, M.D.—Head; Ophthalmology Project, National Institute of Neurological Diseases and Blindness, U.S. Public Health Service, Bethesda, Md.
SCOTT, OWEN—Executive Officer, National Institute of General Medical Sciences, U.S. Public Health Service, Bethesda, Md.
SELIGMAN, ARNOLD M., M.D.—Chairman, Department of Surgery, Sinai Hospital, Baltimore, Md.

SELTSER, RAYMOND, M.D.—The Johns Hopkins University School of Public Health, Baltimore, Md.

SELTZER, CARL C., Ph. D.—Research Associate in Physical Anthropology, Peabody Museum, Harvard University, Cambridge, Mass.

SHAPIRO, HARRY, M.D.—Curator of Anthropology, American Museum of Natural History, New York, N.Y.

SHUBIK, PHILLIPE, M.D.—Professor of Oncology, Chicago Medical School, Chicago, Ill.

SILVETTE, HERBERT, Ph. D.—Visiting Professor of Pharmacology, Medical College of Virginia, Richmond, Va.

SIRKEN, MONROE, Ph. D.—Acting Chief, Division of Health Records, The National Center for Health Statistics, U.S. Public Health Service, Washington, D.C.

SLOAN, MARGARET H., M.D.—Special Assistant to Director, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.

SPIEGELMAN, MORTIMER—Associate Statistician, Metropolitan Life Insurance Company, New York, N.Y.

STALLONES, REUEL, M.D.—University of California School of Public Health, Berkeley, Calif.

STEINBERG, ARTHUR, Ph. D.—Biologist, Professor in Department of Biology, Western Reserve University, Cleveland, Ohio

STEWART, HAROLD L., M.D.—Chief Laboratory of Pathology, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.

STOCKS, PERCY, M.D.—World Health Organization Consultant, Former Chief Medical Statistician in the Office of the General Registrar (1933–50), London, England

STOUT, ARTHUR P., M.D.—Professor Emeritus of Surgery, Laboratory of Surgical Pathology, College of Physicians and Surgeons, Columbia University, New York, N.Y.

STOWELL, ROBERT, M.D., Ph. D.—Scientific Director, Armed Forces Institute of Pathology, Washington, D.C.

SYME, SHERMAN LEONARD—Sociologist, San Francisco Field and Training Station, U.S. Public Health Service Hospital, San Francisco, Calif.

TAEUBER, K. E.—Research Associate, Population Research and Training Center, University of Chicago, Chicago, Ill.

TOBACCO INSTITUTE, INC., Washington, D.C.

TOBACCO INSTITUTE RESEARCH COMMITTEE, New York, N.Y.

TOKUHATA, GEORGE, Ph. D., D.P.H.—Chief of Epidemiology, St. Jude Research Hospital, Institute of Biology and Pediatrics, Memphis, Tenn., and Assistant Professor of Preventive Medicine, University of Tennessee, College of Medicine, Memphis, Tenn.

TOMPSETT, RALPH, M.D.—Professor of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Tex., and Director of Medical Education, Baylor University Medical Center, Dallas, Tex.

TOTTEN, ROBERT S., M.D.—Associate Professor of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

TURNER, CLAUDE C.—Director, Tobacco Policy Staff, Agriculture Stabilization and Conservation Service, United States Department of Agriculture, Washington, D.C.

VINCENT, WILLIAM J.—Student, University of California, Los Angeles, Calif.

VON SALLMANN, LUDWIG, M.D.—Chief, Ophthalmology Branch, National Institute of Neurological Diseases and Blindness, U.S. Public Health Service, Bethesda, Md.

VORWALD, ARTHUR, M.D.—Chairman, Department of Industrial Medicine and Hygiene, Wayne University College of Medicine, Detroit, Mich.

WALKER, C. B., B.A.—Biostatistics Section, Research and Statistics Division, Department of National Health and Welfare, Ottawa, Canada

WALLENSTEIN, MERRILL, Ph. D.—Chief, Physical Chemistry Division, National Bureau of Standards, Washington, D.C.

WEBB, BLAIR M., M.D.—Otolaryngologist and ENT Consultant at the National Institutes of Health, U.S. Public Health Service, Bethesda, Md.

WEINSTEIN, HOWARD I., M.D.—Director, Division of Medical Review, Food and Drug Administration, Washington, D.C.

WOOLSEY, THEODORE D.—Assistant Director, National Center for Health Statistics, U.S. Public Health Service, Washington, D.C.

WYATT, JOHN P., M.D.—Professor of Pathology, St. Louis University School of Medicine, St. Louis, Mo.

ZERZAVY, FRED M., M.D.—Department of Maternal and Child Health, The Johns Hopkins School of Public Health, Baltimore, Md.

ZUKEL, WILLIAM, M.D.—Associate Director, Collaborative Studies, National Cancer Institute, U.S. Public Health Service, Bethesda, Md.

Chapter 6

Chemical and Physical Characteristics of Tobacco and Tobacco Smoke

714-422 O-64—5

Contents

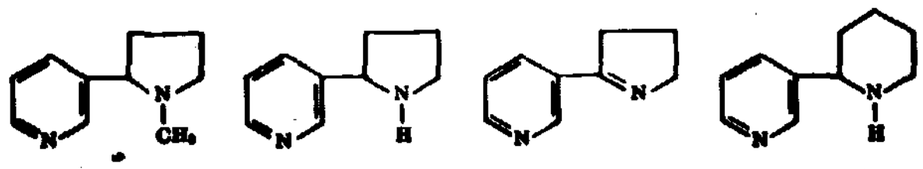
List of Tables

Chapter 6

Tobacco is an herb which man has smoked for over 300 years. The plant was given the generic name *Nicotiana* after Jean Nicot, French ambassador to Portugal, who in 1560 publicly extolled the virtue of tobacco as a curative agent. The species *Nicotiana tabacum* is now the chief source of smoking tobacco and is the only species cultivated in the United States.

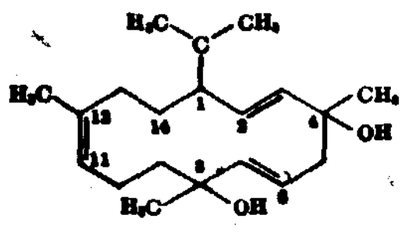
CHEMISTRY OF TOBACCO

The tobacco leaf contains a complex mixture of chemical components: cellulosic products, starches, proteins, sugars, alkaloids, pectic substances, hydrocarbons, phenols, fatty acids, isoprenoids, sterols, and inorganic minerals. Many of the several hundred components isolated have been found to occur also in other plants. Two groups of components are specific to tobacco and have not as yet been isolated from other natural sources. One includes the alkaloid nicotine and the related companion substances nornicotine, myosmine, and anabasine. These nitrogen-containing substances are all

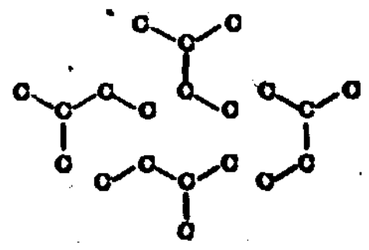


Nicotine Nornicotine Myosmine Anabasine

basic and hence extractable with acid. Seven members of a second group of compounds fairly distinctive to tobacco have been isolated and characterized (1962–63) by D. L. Roberts and R. L. Rowland (36). They are described as isoprenoids, since the structures are divisible into units of isoprene, the building principle of rubber, of the red pigment of the tomato, and of the yellow pigment of the carrot, as illustrated in the following formulas:



Isoprenoid tobacco component



4 Isoprene units

Although none of the 7 isoprenoid components of tobacco has been isolated from another source, the hydrocarbon cembrene from a pine exudate has the same 14-membered ring with the same complement of an isopropyl group at C_1 and methyl groups at C_4, C_8, and C_{12} (9).

COMPOSITI OF CIGARETTE SMOKE

Cigarette smoke is an heterogeneous mixture of gases, uncondensed vapors, and liquid particulate matter (32). As it enters the mouth the smoke is a concentrated aerosol with millions or billions of particles per cubic centimeter (25, 30). The median size of the particles is about 0.5 micron (1). For purposes of investigating chemical composition and biological properties, smoke is separated into a particulate phase and a gas phase, and the gas phase is frequently subdivided into materials which condense at liquid-air tempera- those which do not. The large quantities of material required for investigation of the chemical components are prepared on smoking machines (25) in which large numbers of cigarettes are smoked simultaneously in a fashion designed to simulate average smoking habits, and a yellow-brown condensate known as tobacco tar is collected in traps cooled to the temperature of dry ice (−70° C.) or liquid nitrogen (- 196° C.). The tar thus contains all of the particulate phase of smoke as well as condensable components of the gas phase. The amount of tar from the smoke of one cigarette is between 3 and 40 mg., the quantity varying according to the burning and condensing conditions, the length of the cigarette, the use of a filter, porosity of paper, content of tobacco, weight and kind of tobacco.

An important factor determining the composition of cigarette smoke is the temperature in the burning zone. While air is being drawn through the cigarette the temperature of the burning zone reaches approximately 884° C. and when the cigarette is burning without air being drawn through it the temperature is approximately 835° C_1 (42). The smoke generated during puffing, when air is being drawn through the cigarette, is called main-stream smoke; that generated when the cigarette is burning at rest is called side-stream smoke. At the temperatures cited extensive pyrolytic reactions occur. Some of the many constituents of tobacco are stable enough to distil unchanged, but many others suffer extensive reactions involving oxidation, dehydrogenation, cracking, rearrangement, and condensation. The large number and variety of compounds in tobacco smoke tar is reminiscent of the composition of the tar formed on carbonization of coal, which in many cases is conducted at temperatures lower than those of a burning cigarette It is thus not surprising that some 500 different compounds have been identified in either the particulate phase of cigarette smoke or in the gas phase.

In one study (50) regular cigarettes (70 mm. long, about 1 g. each) without filter tips produced 17–40 mg. of tar per cigarette. In another investigation (43) 174,000 regular size American cigarettes afforded a total of 4 kg. of tar, an average of 23 mg. per cigarette. In still another study (31) 34,000 [redacted] cigarettes were smoked mechanically on a constant puff-volume type machine with which 35-ml. puffs, each of two seconds duration, were taken at one minute intervals from each cigarette. Eight puffs were required to smoke each cigarette to an average butt length of 30 mm. The smoke was condensed in a series of three glass traps cooled in liquid air. The condensate was rinsed out of the traps with ether, water, and hexane. The yield of condensate nonvolatile at 25° C. and 25 mm. of mercury was 20.9 mg. per cigarette.

Procedures for gross separation into basic, acidic, phenolic, and neutral fractions and for further processing of these fractions vary from laboratory to laboratory. The criteria upon which identification is based also vary. The most reliable identifications are based upon an ultraviolet absorption spectrum and/or a fluorescence spectrum in good agreement over the entire range with that of an authentic sample and include one or more of the following: Rf value observed in a paper chromatogram (41); order of elution from alumina; mass spectrometry.

COMPOUNDS OF THE PARTICULATE PHASE OTHER THAN HIGHER POLYCYCLICS

This brief summary is based largely on the comprehensive review by Johnstone and Plimmer of the Medical Research Council at Exeter University, England (24). It should be noted that water constitutes 27 percent of the particulate phase. The major groups of compounds included are shown in Table 1.

Aliphatic and Alicyclic Hydrocarbons

Almost all of the possible hydrocarbons, C_1 through C_{41}, saturated and unsaturated, straight-chain and branched-chain, have been reported to be present in tobacco smoke. Intermediate, normally liquid paraffins are present. All the C_{20} through C_{33} *n*-alkanes have been identified, as well as the C_{27} and C_{29}-C_{33} isoparaffins.

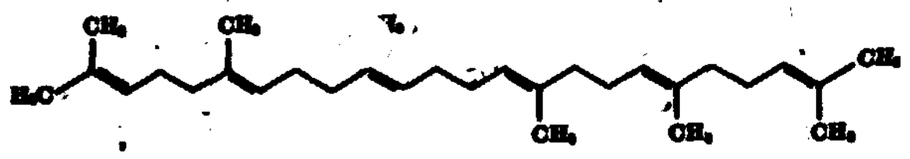
Table 1.—*Major classes of compounds in the particulate phase of cigarette smoke*

Class	Percent in particulate* phase	Number of compounds	Toxic action on lung
Acids	7.7-12.6	25	Some irritant
Glycerol, glycol, alcohols	5.5-6.3	18	Possible irritation
Aldehydes and ketones	6.3	21	Some irritant
Aliphatic hydrocarbons	4.9	64	Some irritant
Aromatic hydrocarbons	6.44	81	Some carcinogenic
Phenols	1.0-3.6	45	Irritant and possibly cocarcinogenic
	69%	254	

*Water 27%.

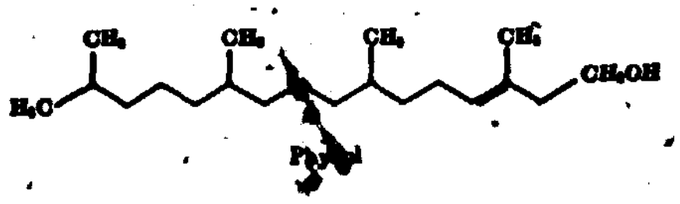
Terpenes and Isoprenoid Hydrocarbons

Isoprene, the basic unit of the terpenes and of higher terpenoids has been identified in cigarette smoke (34) as have its dimers, dipentene and 1,8-*p*-menthadiene. The triterpene squalene, consisting of six isoprene units and shown to be present in smoke (47) is of interest because of the possibility of its being cyclized to polycyclic compounds and because of its ready



Squalene

reaction with air to form hydroperoxides (which would be destroyed during attempted isolation); a hydroperoxide derived from cholesterol has been shown to be carcinogenic (cancer-causing), at least under certain conditions of administration (12). Phytadienes, products of the dehydration of the diterpene alcohol phytol, are also present in smoke and subject to air oxidation to hydroperoxides.



Alcohols and Esters

A wide variety of mono- and dihydric alcohols, both aliphatic and aromatic, are present in tobacco smoke. Solanesol, a primary alcohol containing 9 isoprene units, has been found in both tobacco and tobacco smoke; 20 g. of pure material was isolated from 10 lbs. of flue-cured aged tobacco (0.44 percent). Grossman et al (13) found that pyrolysis of solanesol at 500° C. gives isoprene, its dimer dipentene, and other terpenoid products and concluded that the alcohol is the source of terpenoid compounds which are important factors in the flavor of tobacco smoke.

Ethylene glycol and glycerol have been found present in smoke, but it is not clear from the literature whether they are present in smoke from untreated tobacco or arise from addition of these humectant substances to tobacco to improve moistness.

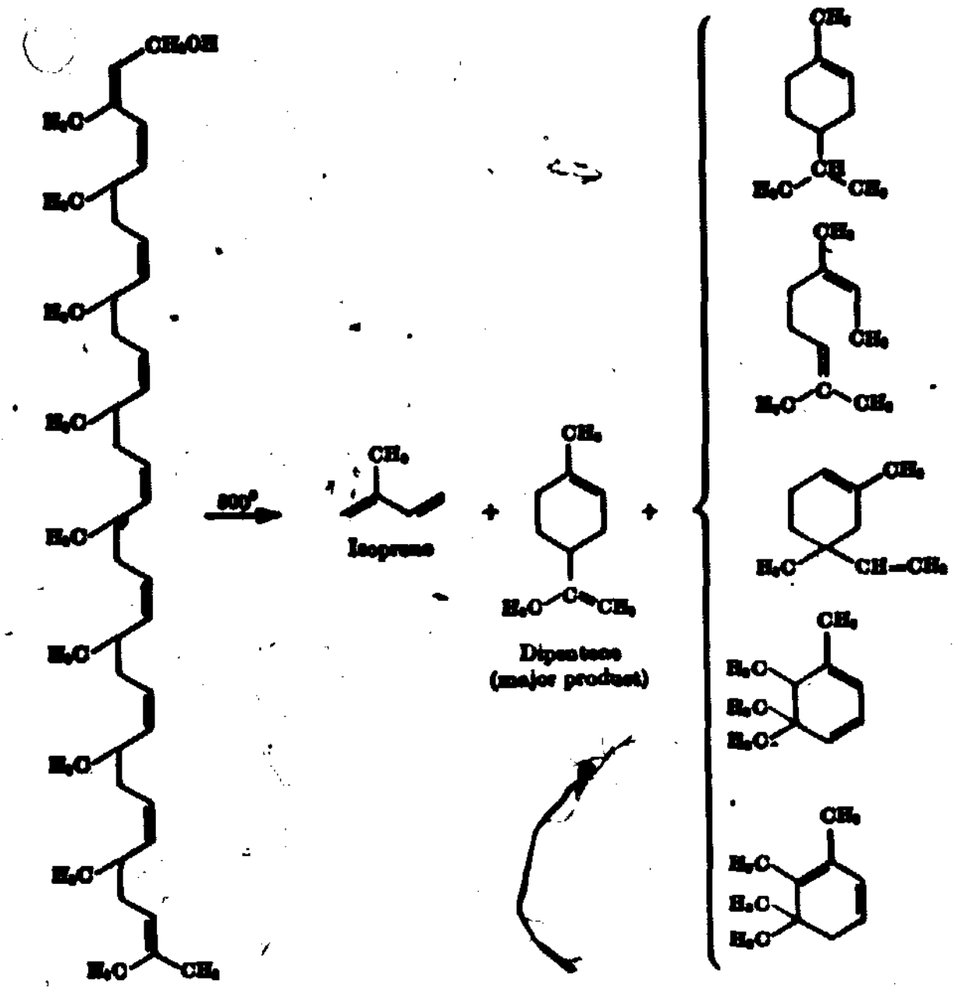
Many common esters, such as the ethyl esters of the C_2, C_3, and C_4 fatty acids, are present in smoke. Higher fatty acids are found both as free acids and as esters.

Sterols

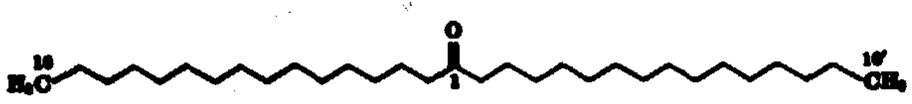
Stigmasterol, β-sitosterol, and γ-sitosterol have been isolated from tobacco smoke. Indeed the sterol fraction is reported (29) to constitute approximately 0.15 percent of whole tar. The sterols are of interest as possible precursors of polycyclic aromatic hydrocarbons and because of the evidence, noted above, that sterol hydroperoxides can be carcinogenic.

Aldehydes and Ketones

Most common aldehydes of low molecular weight (acetaldehyde, propionaldehyde, acetone, methyl ethyl ketone, etc.) have been found present



in tobacco smoke, as have such dicarbonyl compounds as glyoxal and diacetyl. Dipalmityl ketone exemplifies ketones of high molecular weight isolated from tobacco smoke.



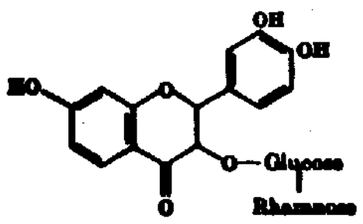
Dipalmityl ketone

Acids

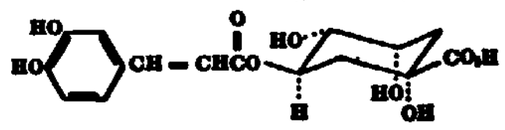
A large number of volatile and nonvolatile acids of low molecular weight are present in tobacco smoke. Fatty acids of chain length C_{12} to C_{18} are reported to constitute 1 percent of the whole tar and the bulk of these acids are present in the free form (46). Unsaturated fatty acids and keto acids (e.g., pyruvic acid) are also present.

Phen and Polyphenols

Since the phenols and polyphenols present in tobacco leaf play an important role in the curing and smoking quality of tobacco, a great deal of investigative work has been done on the estimation, separation, and identification of complex tobacco phenols such as rutin and chlorogenic acid. The presence of simple phenols in tobacco smoke was established as early as 1871. The phenol content of smoke became of increasing importance with



Rutin

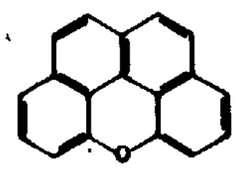


Chlorogenic acid

the demonstration that phenol and substituted phenols can function as cocarcinogens; that is, they promote the appearance of skin tumors in mice following application of a single initiating dose of a known carcinogen (4). Furthermore, the smoke from one cigarette contains as much as 1 mg. of phenols (7). In addition to simple alkylphenols, naphthols, and the polyphenols, resorcinol and hydroquinone are also present.

Alkaloids, Nitrogen Bases, and Heterocyclics

Pyridine, nicotine, nornicotine, and other substituted pyridine bases constitute some 8–15 percent of whole tar; nicotine and nornicotine constitute about 7–8 percent of the total tar. The companion bases are products of the pyrolysis of the alkaloids present in tobacco leaf. Quinoline and three polycyclic heterocyclic compounds have also been identified in smoke (45) and will be discussed later since the three polycyclic compounds are carcinogenic. A pentacyclic compound related to xanthene, namely 1,8,9-perinaphthoxanthene, has been identified in smoke (45).



1,8,9-Perinaphthoxanthene

Amino Acids

Although tobacco leaf contains a number of amino acids, relatively few have been found present in smoke; among these are glutamine and glutamic acid.

Inorganic Components

It is estimated that the main-stream smoke from one cigarette contains about 150 μg. of metallic constituents, which are mainly potassium (90 percent), sodium (5 percent), and traces of aluminum, arsenic, calcium, and copper. Arsenic is reported to be present to the extent of 0.3–1.4 μg. in the smoke of one cigarette. The inorganic compounds are most likely chlorides, but metals themselves may be present.

Apparently beryllium is present in tobacco in trace quantities, but is not volatilized in the smoking process (48). Nickel is present in cigarettes in trace amounts and may occur in main-stream smoke to a small extent, probably as the chloride (31). Spectrographic analysis has shown the presence of chromium in smoke at a level of less than 0.06 μg. per cigarette. This level appears too low to represent a hazard (48).

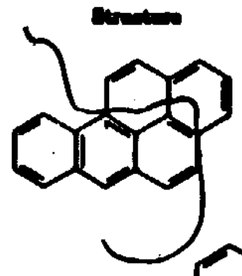
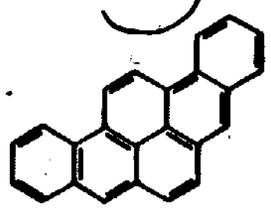
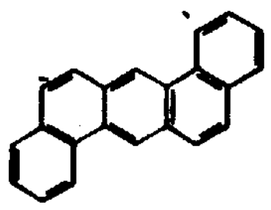
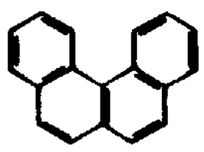
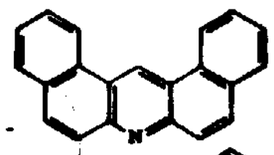
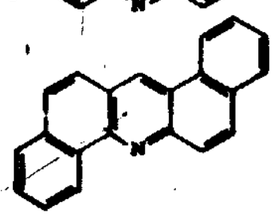
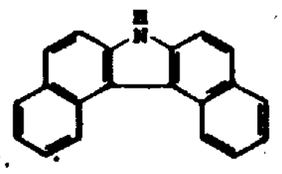
Noncarcinogenic Aromatic Hydrocarbons

The aromatic hydrocarbons present in tobacco smoke have received an enormous amount of attention since some of them are carcinogenic. Noncarcinogenic hydrocarbons of smoke containing one to three rings include benzene, toluene and other alkylbenzenes, acenaphthene, acenaphthylene, fluorene, anthracene, and phenanthrene. Hydrocarbons of established carcinogenicity to mice all contain from four to six condensed rings. However, no less than 27 hydrocarbons containing four or more condensed rings which have been tested for carcinogenicity with negative results have been isolated from tobacco smoke tar. As methods of separation and identification improve, it is almost certain that additional hydrocarbons will be found present in smoke, because almost every conceivable ring system has been demonstrated to be present and the number of possible alkylated polycyclics is very large indeed.

CARCINOGENIC HYDROCARBONS AND HETEROCYCLICS IN TOBACCO SMOKE

In 1925–30 Kennaway et al. in seeking to identify the active substance in high-boiling fractions of coal tar distillates of established carcinogenicity to mice, discovered that dibenzo(a,h)anthracene (for formula, see Table 2) prepared by synthesis evokes skin cancer when applied to the skin of mice (11). The hydrocarbon was recognized as different from the carcinogen of coal tar because its fluorescent spectrum did not match the characteristic three-banded spectrum of the tars. In 1933 Cook and co-workers (11) isolated the coal tar constituent responsible for the characteristic fluorescence and identified it as benzo(a)pyrene. It is one of the most potent of all the carcinogens now known.

TABLE 2.—*Carcinogenic cyclic Compounds Isolated From Cigarette Smoke*

Compound	Structure	Carcinogenicity	Amount reported, ng/1000 cigarettes
1. Benzo(a)pyrene		++++	16 (ave. of 10 reports)
2. Dibenzo(a,l)pyrene		++++	0.02-10 (2 reports)
3. Dibenzo(a,h)anthracene		++	4 (1 report)
4. Benzo(c)phenanthrene		+	not stated
5. Dibenzo(a,j)acridine		+	2.7 (1 report)
6. Dibenzo(a,h)acridine		+	0.1 (1 report)
7. 7H-Dibenzo(c,g)carbazole		+	0.7 (1 report)

Since the discovery of carcinogenic hydrocarbons, a large number of polycyclic hydrocarbons and heterocyclic analogs have been tested for carcinogenicity to mice and to rats in many laboratories, both by application to the skin and by subcutaneous injection. Bioassays in different laboratories, often on independently prepared samples, are remarkably consistent and place a series of hydrocarbons in the same relative order of potency. A compilation (and its supplement) prepared by J. L. Hartwell (16) of the National Cancer Institute lists 2108 compounds of which 481 were reported to cause malignant tumors in animals. All but one of the polycyclic hydrocarbons listed in Table 2 as having been identified in tobacco smoke have already been documented in the Hartwell report and can be assigned a rating as very potent (+ + + +), potent (+ + +), moderately carcinogenic (+ +), or weakly carcinogenic (+) (31). Many other such compounds studied are reported in the Hartwell survey and in another by Arthur D. Little, Inc. (31). The rating assigned to dibenzo(a,i) pyrene is based on experiments with over 10,000 inbred mice in which one subcutaneous injection in the groin of 0.5 mg. of hydrocarbon in tricaprylin produced 50 percent sarcomas at the injection site in 14 weeks and 98 percent tumors in 24 weeks (20). Benzo(a)pyrene is one of the two most potent of the seven carcinogens detected in tobacco smoke and it is present in much larger quantity than any of the other carcinogens listed. Two polycyclic hydrocarbons isolated from tobacco smoke but not yet adequately tested for carcinogenicity are: benzo(j)fluoranthene and dibenzo(a,l)pyrene.

Identification of benzo(a)pyrene is reported in 19 separate investigations; the amount given in the table per 1000 cigarettes (70 mm. long, weighing about 1.0 g. each) is the average of 10 values selected on the basis of the quality of criteria used for identification (31). Compounds 1, 2, 3, 4, and benzo(j)fluoranthene were identified in one laboratory over a period of years and are listed together in a review by Van Duuren (44). Isolation of the three heterocyclic carcinogens (5,6,7) is reported by Van Duuren (45).

Because of losses in the process of fractionation and purification, the amount of carcinogens reported in a given investigation may be less than the amount actually present. Wynder and Hoffman (50) investigated this point by adding a known amount of radioactive C^{14}-labelled benzo(a)pyrene to a smoke condensate and applied the usual procedure for isolation of benzo(a)pyrene, which involved, in the last stages, chromatographing twice on silica gel and four times on paper. The activity of the benzo(a)pyrene finally isolated indicated a loss of 35–40 percent of carcinogen during processing. The amount of benzo(a)pyrene given in Table 2 thus should be multiplied by a factor of 1.5 to give the estimated true amount. Probably the amounts of the other carcinogens in smoke are also at least 1.5 times the reported amounts.

Relatively little work has been done on the components of smoke produced with cigars and pipes. Table 3 summarizing a comparative study made in one laboratory (5) indicates that the amount of benzo(a)pyrene, the only carcinogen in the group studied, increases sharply from cigarettes to cigars to pipes.

TABLE 3.—*Polycyclic hydrocarbons isolated from tobacco smoke*

[μg. per 1000 g. of tobacco consumed]

Hydrocarbon	Cigarettes	Cigars	Pipes
Benzo(a)pyrene	9	34	50
Anthracene	160	110	1,160
Pyrene	126	170	720

COCARCINOGENS

Assays of tobacco smoke tars for carcinogenicity are done by applying a dilute solution of tar in an organic solvent with a camel's hair brush to the backs of mice beginning when the animals are about six weeks old. Application is repeated three times a week for a period of a year or more. The results of a number of such assays present a puzzling anomaly: the total tar from cigarettes has about 40 times the carcinogenic potency of the benzo(a)pyrene present in the tar. The other carcinogens known to be present in tobacco smoke are, with the exception of dibenzo(a,i)pyrene, much less potent than benzo(a)pyrene and they are present in smaller amounts. Apparently, therefore, the whole is greater than the sum of the known parts (27, 33, 49).

One possible or partial explanation of the discrepancy is that the tar contains compounds which, although not themselves carcinogenic, can enhance the cancer-producing properties of the carcinogens. Berenblum and Shubik (3), reporting on cocarcinogenesis, described the potentiating effect of croton oil, which itself is noncarcinogenic except in certain strains of mice (4a), on the action of hydrocarbon carcinogens. Phenol is reported to have a similar potentiating effect (4, 50) and, as noted above, cigarette smoke contains considerable phenolic material. Long chain fatty acid esters (39) and free fatty acids (19) have been shown to function as cocarcinogens, and substances of both types occur abundantly in tobacco smoke. It is possible that the potentiating action of croton oil is due to the presence of fatty acids and their esters. A further observation of possible importance is that some polycyclic hydrocarbons, though very weak or inactive as carcinogens, are capable of initiating malignant growth under the influence of a promoter. Thus benz(a)anthracene, identified in cigarette smoke, is very weak or inactive in initiating malignant growth by itself, but initiates carcinogenesis under the influence of croton oil as promoter (15).

If more were known about the possible cocarcinogenicity of the many inactive components of tobacco smoke, some of the apparent discrepancy between isolation and bioassay data might disappear. It is possible that some of the carcinogenicity of smoke is due to hydroperoxides formed from unsaturated smoke components and destroyed in the isolation procedures. Furthermore both sets of data are far from precise; for example, one estimate of the amount of the highly potent dibenzo(a,i)pyrene per 1000 cigarettes (Table 2) is 0.02μg. and another is 10μg.

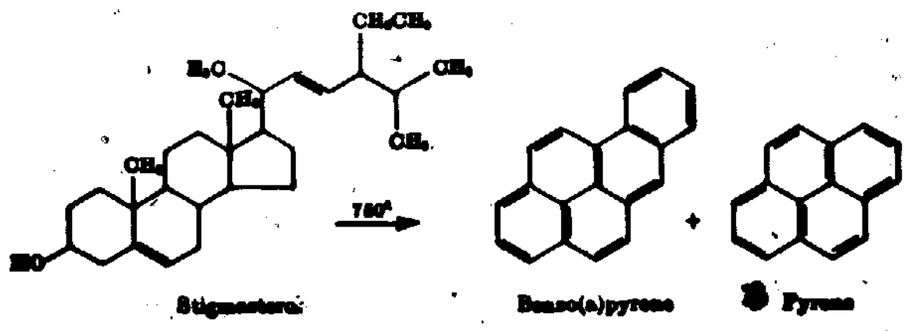
However, it is not necessary to wait for an exact balance of the two sets of data to draw a conclusion from each. The isolation experiments, taken

ne, indicate that cigarette smoke contains a number of identified chemicals which are carcinogenic to mice. The bioassays suggest that cigarette smoke probably contains components which, acting in a manner as yet undescribed, are involved in the induction of tumors in mice.

Assessment of all conceivable synergistic effects presents a gigantic problem for exploration. Tobacco smoke contains considerable amounts of phenols and fatty acids, both of which, as previously mentioned, enhance the activity of known carcinogens. Cellulose acetate filters now in use remove 70–80 percent of acidic constituents of tobacco smoke.

MECHANISM OF THE FORMATION OF CARCINOGENS

Most of the carcinogenic compounds identified in cigarette smoke tar are not present in the native tobacco leaf but are formed by pyrolysis at the high burning temperature of cigarettes. Van Duuren (44) reports formation of benzo(a)pyrene and pyrene on pyrolysis of stigmasterol, a smoke component.



Similar pyrolysis of pyridine or of nicotine gives dibenzo(a,j) acridine and dibenzo(a,h)acridine, both of which are carcinogenic (Table 2). Pyrolysis of nontobacco cigarettes made from vegetable fibers and spinach resulted in formation of benzo(a)pyrene (50).

Hurd and co-workers (22) by careful experimentation have elaborated plausible mechanisms for the formation of polycyclic aromatics by pyrolysis of materials of low molecular weight at temperatures in the range 800–900° C. Postulated radical intermediates are:

(a) $CH_2-O-\dot{C}H \longleftrightarrow \dot{C}H_2-O=CH$

(b) $\ddot{C}H-CH=\dot{C}H \longleftrightarrow \dot{C}H-\ddot{C}H-\ddot{C}H$

(c) $\dot{C}H-CH-CH-\dot{C}H$

These radicals can arise from propylene, toluene, picoline, or pyridine. A variety of polycyclic hydrocarbons can be generated by reaction of these radicals with themselves or with other small radicals present in the heating zone. For example, dimerization of (b) should give benzene.

It thus appears that the pyrolysis of many organic materials can lead to the formation of components carcinogenic to mice. Cigarette paper consists essentially of cellulose. Pyrolysis of cellulose has been shown to produce benzo(a)pyrene. The observation (2) that treatment of tobacco with copper nitrate decreases the benzo(a)pyrene content of the cigarette smoke suggests a possibility for improvement by the use of additives or catalysts. The fact that side-stream smoke contains three times more benzo(a)pyrene than main-stream smoke has been cited (50) as evidence that more efficient oxidation could conceivably lower the content of carcinogenic hydrocarbons.

THE GAS PHASE

The gas phase accounts for 60 percent of total cigarette smoke. Hobbs et al. (34, 35) found that 98.9 mole percent of the gas phase is made up of the following seven components:

Nitrogen	73 mole percent
Oxygen	10
Carbon-dioxide	9.5
Carbon-monoxide	4.2
Hydrogen	1.
Argon	0.6
Methane	0.6
	98.9

The approximately one percent of the gas phase not accounted for by the seven major constituents contains numerous compounds, no less than 43 of which have been identified as present in trace amounts. Some of these are listed in Table 4 (1).

TABLE 4.—*Some gases found in cigarette smoke*

Compound	Concentration	Safe level for industrial exposure*	Toxic action on lung
	(ppm)	(ppm)	
Carbon Monoxide	42,000	100	Unknown
Carbon Dioxide	93,000		None
Methane, ethane, propane, butane, etc.	87,000	500	None
Acetylene, ethylene, propylene, etc.	31,000	5,000	None
Formaldehyde	20	5	Irritant
Acetaldehyde	3,200	200	Irritant
Acrolein	150	0.5	Irritant
Methanol	700		Irritant
Acetone	1,100	200	Irritant
Methyl ethyl ketone	300	200	Irritant
Ammonia	300	100	Irritant
Nitrogen Dioxide	250	5	Irritant
Methyl Nitrite	200		Unknown
Hydrogen Sulfide	40	20	Irritant
Hydrogen Cyanide	1,000	10	Respiratory enzyme poison
Methyl Chloride	1,200	100	Unknown

*The values listed refer to time-weighted average concentrations for a normal work day.

EFFECTS ON CILIARY ACTIVITY*

An important line of investigation was opened up by the report by Hilding (18) that cigarette smoke is capable of inhibiting the transport activity of ciliated cells such as found in the respiratory tract. It has been suggested (10, 17) that failure of ciliary function to provide a constantly moving stream of mucus enables environmental carcinogens to reach the epithelial cells. Kensler and Battista (28) describe development of a method of bioassay for inhibition of ciliary transport activity involving exposure of the trachea of a rabbit to the test material. The smoke from a regular cigarette was found to inhibit transport activity by 50 percent after exposure to two or three puffs. Several commercial filter cigarettes gave essentially the same result. The fact that these filters lower the phenol content by 70 to 80 percent and trap about 40 percent of the particulate phase suggested that neither phenolic nor particulate materials are responsible for the inhibition noted. The next trial was with an absolute filter, that is, one which removes the entire particulate phase and gives nonvisible gas. The observation that such treatment did not significantly alter the inhibitory effect of the puff established that components of the gas phase are responsible for inhibition of ciliary transport activity. Assays of known components of the gas phase showed the following compounds to possess such activity: hydrogen cyanide, formaldehyde, acetaldehyde, acrolein, and ammonia, although no one of the [illegible] at levels high enough to produce the effect noted for smoke.

Activated carbons di[illegible]arkedly in their adsorption characteristics. Carbon filters previously employed in cigarettes do not have the specific power to scrub the gas phase. It has been reported that a filter containing special carbon granules removes gaseous constituents which depress ciliary activity (28).

PESTICIDES AND ADDITIVES

Before 1930 practically the only insecticides used in the growing of tobacco were lead arsenate and paris green (the mixed acetate-arsenite salt of copper). Analysis of 6 brands of American cigarettes purchased in 1933 showed a range of 7.5–26.4 parts of As_2O_3 per million, with an average value of 13.9 ppm. (6). Cogbill and Hobbs (8) found that main-stream smoke of cigarettes containing 7.1 μg. of arsenic per cigarette contains 0.031 μg. per puff. This amount would be equivalent to 0.25 μg. of arsenic per cigarette (8 puffs), and hence a smoker consuming 2.5 packs of such cigarettes per day might inhale 12.5 μg. of arsenic per day. By comparison, analysis of the atmosphere of New York City over a 12-year period indicated an average content of 100–400 μg. of arsenic per 10 cubic meters, which is an approximate daily intake per person (38).

Extensive Federal efforts to discourage the use of arsenicals for the control of tobacco hornworms on the growing tobacco crop resulted in a sharp de-

*This topic is discussed more fully in Chapter 10.

cline in the arsenic cont of cigarettes after 1950. Thus, the average arsenic content of 17 brands of cigarettes analyzed in 1958 was 6.2 ppm. of As_2O_3 (14).

It seems unlikely that the amount of arsenic derived even from unfiltered cigarettes is sufficient to present a health hazard.

Chemicals recommended by the Department of Agriculture for the control of tobacco insects are: malathion, parathion, Endosulfan, DDT, TDE, endrin, dieldrin, Guthion, aldrin, heptachlor, Diazinon, Dylox, Sevin, and chlordane (42a). Trace amounts of TDE and endrin have been detected in commercial cigarettes and cigarette smoke. Guthion and Sevin residues were detected in main-stream cigarette smoke at levels approximating 0.3 percent and 1 percent of that added to cigarettes prior to smoking. Tobacco treated with Guthion and Sevin at the recommended levels showed no measurable contamination of main-stream cigarette smoke (4b). (For discussion of carcinogenicity of tobacco pesticides, see Chapter 9.)

Cigarette manufacture in the United States includes use of additives such as sugars, humectants, synthetic flavors, licorice, menthol, vanillin, and rum. Glycerol and methylglycerol are looked on with disfavor as humectants because on pyrolysis they yield the irritants acrolein and methylglyoxal. Additives have not been used in the manufacture of domestic British cigarettes since the Customs and Excise Act of 1952, Clause 176, and probably longer, inasmuch as Section 5 of the Tobacco Act of 1842 imposed a widespread prohibition on the use of additives in tobacco manufacture.

SUMMARY

Of the several hundred compounds isolated from the tobacco leaf, two groups are specific to tobacco. One of these groups includes the alkaloid nicotine and related substances. The other includes compounds described as isoprenoids. Cigarette smoke is an heterogeneous mixture of gases, uncondensed vapors, and particulate matter. In investigating chemical composition and biological properties, it is necessary to deal separately with the particulate phase and gas phase of smoke.

Components of the particulate phase other than the higher polycyclics include aliphatic and alicyclic hydrocarbons, terpenes and isoprenoid hydrocarbons, alcohols and esters, sterols, aldehydes and ketones, acids, phenols and polyphenols, alkaloids, nitrogen bases, heterocyclics, amino acids, and inorganic chemicals such as arsenic, potassium, and some metals. Seven polycyclic compounds isolated from cigarette smoke have been established to be carcinogenic. They are shown in Table 2. The over-all carcinogenic potency of tobacco tar is many times the effect which can be attributed to substances isolated from it. The difference may be associated in part with the presence in tobacco smoke of cocarcinogens, several of which have been identified as smoke components.

Components of the gas phase of cigarette smoke have been shown to produce various undesirable effects on test animals or organs, one of which is suppression of ciliary transport activity in trachea and bronchi.

REFERENCES

1. Albert, R. E., Nelson, N. Special report to the Surgeon General's Advisory Committee on Smoking and Health.
2. Alvord, E. T., Cardon, S. Z. The inhibition of formation of 3,4-benzpyrene. Brit J Cancer 10: 498–506, 1956.
3. Berenblum, I., Shubik, P. The role of croton oil applications, associated with a single painting of a carcinogen, in tumour induction of the mouse skin. Brit J Cancer 1: 379–82, 1947.
4. Boutwell, R., Bosch, D. K. The tumor-promoting action of phenol and related compounds for mouse skin. Cancer Res 19: 413–24, 1959.
4a. Boutwell, R., Bosch, D. K., and Rusch, H. P. On the role of croton oil in tumor formation. Cancer Res 17: 71, 1957.
4b. Bowery, T. G., Guthrie, F. E. Determination of insecticide residues on green and flue-cured tobacco and in main-stream cigarette smoke. Agriculture and Food Chem 9(3): 193–7, 1961.
5. Campbell, J. M., Lindsey, A. J. Polycyclic hydrocarbons in cigar smoke. Brit J Cancer 11: 192–5, 1957.
6. Carey, F. P., Blodgett, G., Satterlee, H. S. Preparation of samples for determination of arsenic. Oxygen-bomb combustion method. Industr Eng Chem Anal Ed 6, 327–30, 1934.
7. Clemo, G. R. Some aspects of the chemistry of cigarette smoke. Tetrahedron 3: 168–74, 1958.
8. Cogbill, E. C., Hobbs, M. E. Transfer of metallic constituents of cigarets to the main-stream smoke. Tobacco Sci 1: 68–73, 1957.
9. Dauben, W. G., Thiessen, W. E., Resnick, P. R. Cembrene, A 14-membered ring diterpene hydrocarbon. J Amer Chem Soc 84: 2015–6, 1962.
10. Falk, H. L., Tremer, H. M., Kotin, P. Effect of cigarette smoke and its constituents on ciliated mucus-secreting epithelium. J Nat Cancer Inst 23: 999–1012, 1959.
11. Fieser, L. F., Fieser, M. Topics in organic chemistry. New York, Reinhold, 1963, p. 43–56.
12. Fieser, L. F., Greene, T. W., Bischoff, F., Lopez, G., Rupp, J. J. [Communication to the editor] A carcinogenic oxidation product of cholesterol. J Amer Chem Soc 77: 3928–9, 1955.
13. Grossman, J. D., Deazyck, E. J., Ikeda, R. M., Bavley, A. A study of pyrolysis of solanesol. Chem Industr 1950–1962.
14. Guthrie, F. E., McCants, C. B., Small, H. G., Jr. Arsenic content of commercial tobacco, 1917–1958. Tobacco Sci 3: 62–4, 1959.
15. Hadler, H. I., Darchun, V., Lee, K. Initiation and promotion activity of certain polynuclear hydrocarbons. J Nat Cancer Inst 23: 1383–7, 1959.
16. Hartwell, J. L. Survey of compounds which have been tested for carcinogenic activity, Federal Security Agency, Public Health Service Pub No. 149, 1951. 583 p.
17. Hilding, A. C. On cigarette smoking, bronchial carcinoma and ciliary action. 3. Accumulation of cigarette tar upon artificially produced

deciliated islands in respiratory epithelium. Ann Othol 65: 116–30, 1956.

18. Hilding, A. C. On cigarette smoking, bronchial carcinoma and ciliary action. 2. Experimental study on the filtering action of cow's lungs, the deposition of tar in the bronchial tree and removal by ciliary action. New Eng J Med 254: 1155–60, 1956.
19. Holati, P. Tumor promoting effects of some long chain fatty acids in experimental skin carcinogenesis in the mouse. Acta Path Microbiol Scand 46: 51–8, 1959.
20. Homburger, F., Tregier, A. Modifying factors in carcinogenesis. Progr Exp Tumor Res 1: 311–28, 1960.
21. Hurd, C. D., Macon, A. R. Pyrolytic formation of arenes. 4. Pyrolysis of benzene, toluene and radioactive toluene. J Amer Chem Soc 84: 4524–6, 1962.
22. Hurd, C. D., Macon, A. R., Simon, J. I., Levetan, R. V. Pyrolytic formation of arenes. 1. Survey of general principles and findings. J Amer Chem Soc 84: 4509–15, 1962.
23. Hurd, C. D., Simon, J. I. Pyrolytic formation of arenes. 3. Pyrolysis of pyridine, picolines and methylpyrazine. J Amer Chem Soc 84: 4519–24, 1962.
24. Johnstone, R. A. W., Plimmer, J. R. The chemical constituents of tobacco and tobacco smoke. Chem Rev 59: 885–936, 1959.
25. Keith, C. H., Newsome, J. R. Quantitative studies on cigarette smoke. 1. An automatic smoking machine. Tobacco 144: (13) 26–32, May 29, 1957.
26. Keith, C. H., Newsome, J. R. Quantitative studies on cigarette smoke. 2. The effect of physical variables on the weight of smoke. Tobacco 144 (14): 26–31, Apr 5, 1957.
27. Kennaway, E., Lindsey, A. J. Some possible exogenous factors in the causation of lung cancer. Brit Med Bull 14: 124–31, 1958.
28. Kensler, C. J., Battista, S. P. Components of cigarette smoke with ciliary-depressant activity. New Eng J Med 269: 1161–1166, 1963.
29. Kosak, A. I., Swinehart, J. S., Taber, D., Van Duuren, B. L. Stigmasterol in cigarette smoke. Science 125: 991–2, 1957.
30. Langer, G., Fisher, N. A. Concentration and particle size of cigarette-smoke particles. AMA Arch Industr Health 13: 372–8, 1956.
31. Liggett & Myers Tobacco Co. Arthur D. Little, Inc. Special report to the Surgeon General's Advisory Committee on Smoking and Health.
32. Lindsey, A. J. Some observations upon the chemistry of tobacco smoke. In: James, G., Rosenthal, T., eds. Tobacco and health. Springfield, Ill., Thomas, 1962. Chapter 2, p. 21–32.
33. Orris, L., Van Duuren, B. L., Kosak, A. I., Nelson, N., Schmitt, F. L. The carcinogenicity for mouse skin and the aromatic hydrocarbon content of cigarette-smoke condensates. J Nat Cancer Inst 21: 557–61, 1958.
34. Osborne, J. S., Adamek, S., Hobbs, M. E. Some components of gas phase of cigaret smoke. Anal Chem 28: 211–5, 1956.

35. Philippe, R. J., Hobbs, M. E. Some components of the gas phase of cigaret smoke. Anal Chem 28: 2002–6, 1956.
36. Roberts, D. L., Rowland, R. L. Macrocyclic diterpenes a and B–4, 8, 13-Duvatriene –1,3—diols from tobacco. J Org Chem 27: 3989–95, 1962.
37. Rowland, R. L., Rodgman, A., Schumacher, J. N., Roberts, D. L., Cook, U. O., Walker, W. E. 1963 (In press).
38. Satterlee, H. S. The problem of arsenic in American cigarette tobacco. New Eng J Med 254: 1149–54, 1956.
39. Setala, H. Tumor promoting and co-carcinogenic effects of some non-ionic lipophilic—hydrophilic (surface active) agents. Acta Path Microbiol Scand (Suppl No. 115) p. 1–93, 1956.
40. Shubik, P., Hartwell, J. L. Supplement 1, Department of Health, Education and Welfare, PHS Pub No. 149, 1957.
41. Tarbell, D. S., Brooker, E. G., Vanderpool, A., Conway, W., Claus, C. J., Hall, T. J. A system for paper chromotography of 3,4-benzpyrene, some derivates and other polycyclic aromatic hydrocarbons. J Amer Chem Soc 77: 767–8, 1955.
42. Touey, G. P., Mumpower, R. C. Measurement of the combustion-zone temperature of cigarettes. Tobacco 144: (8) 18–22, Feb 22, 1957.
42a. U.S. Department of Agriculture. Insecticide recommendations of the Entomology Research Division for the Control of Insects Attacking Crops and Livestock for 1963. Handbook No. 120, Agricultural Research Service and Federal Extension Service, 1963.
43. Van Duuren, B. L. Identification of some polynuclear aromatic hydrocarbons in cigarette smoke condensate. J Nat Cancer Inst 21: 1–16, 1958.
44. Van Duuren, B. L. Some aspects of the chemistry of tobacco smoke. In: James, G., Rosenthal, T. eds. Tobacco and health. Springfield, Ill., Thomas, 1962. Chapter 3, p. 33–47.
45. Van Duuren, B. L. The polynuclear aromatic hydrocarbons in cigarette smoke condensate. 2. J Nat Cancer Inst 21: 623–30, 1958.
46. Van Duuren, B. L., Schmitt, F. L. Isolation and identification of some components of cigarette smoke condensate. J Org Chem 23: 473–5, 1958.
47. Van Duuren, B. L., Schmitt, F. L. Isolation and identification of squalene from cigarette smoke condensate. Chem Industr, 1006–7, 1958.
48. Williams, J. F., Garmon, R. G. Beryllium in cigaret tobacco. Tobacco Sci 5: 25–7, 1961.
49. Wynder, E. L., Fritz, L., Furth, N. Effect of concentration of benzopyrene in skin carcinogenesis. J Nat Cancer Inst 19: 361–70, 1957.
50. Wynder, E. L., Hoffmann, D. Present status of laboratory studies on tobacco carcinogenesis. Acta Path Microbiol Scand 52: 119–32, 1961.
51. Wynder, E. L., Hoffman, D. A study of tobacco carcinogenesis. 7. The role of higher polycyclic hydrocarbons. Cancer 12: 1079–86, 1959.